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14. ABSTRACT
We obtained polymeric nanoparticles containing redox-sensitive moieties, which can be removed by chemically or enzymatically triggered reduction. Since the trimethyl lock-based functional groups undergo the triggered reduction to release a lactone and expose free hydrophilic functional groups, a class of trimethyl lock chemistry-based monomers were synthesized and polymerized to obtain biodegradable polymers for the redox-sensitive nanoparticles. Nanoparticles were prepared from the redox-sensitive biodegradable polymers via an emulsion method. The dynamic light scattering (DLS) experiment ensured that the size of nanoparticle significantly changed upon chemical reduction with sodium dithionite. In addition, the drug incorporated nanoparticles released the drug by the chemical reduction with sodium dithionite and the released hydrophobic drug was quantitatively profiled by HPLC. The nanoparticles that are able to respond to the triggered reduction would be promising as a drug delivery platform to target tumors.

15. SUBJECT TERMS
Redox-sensitive, Nanoparticles, Targeted Drug Delivery, Breast cancer, Hypoxia

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Hypoxia-sensitive, multifunctional nanoparticles for targeted drug delivery to breast cancer

Hanjoung Cho, Jungeun Bae, Vivek K. Garripelli, Seongbong Jo

Introduction

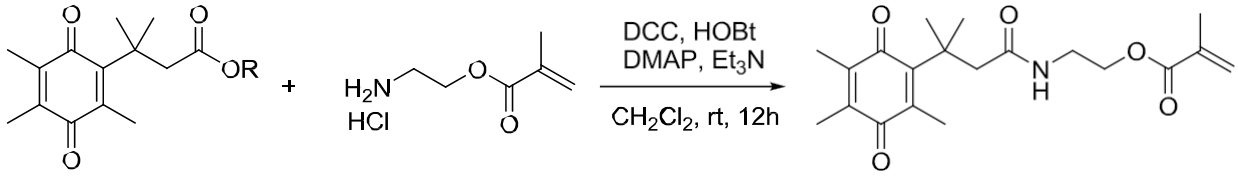
A novel multifunctional nanoparticle is proposed for highly selective drug delivery to breast cancer via dual-targeting of hypoxia and angiogenesis in tumor. Breast cancer would be synergistically inhibited with the multifunctional nanoparticle since the dual-targeted drug delivery can overcome dose-limiting systemic toxicity of cancer drugs as well as hypoxia-induced resistance. Bioresponsive polymeric nanoparticles have been sought for the development of a multifunctional nanoparticle drug delivery system selectively targeting tumor redox state. A novel redox-sensitive biodegradable polymer with "trimethyl lock" quinone was synthesized for the preparation of redox-sensitive nanoparticles to carry paclitaxel. The redox-sensitive nanoparticles were characterized and evaluated for drug delivery application.

Body

Synthesis of methacryl monomers for nanoparticle preparation

It has been established that the trimethyl-locked benzoquinone spontaneously transforms into lactone via two electron reduction triggered by chemicals or reductive enzymes.[1,2] Chemical reduction-induced shedding of trimethyl-locked quinone from dendrimer and liposomes has been demonstrated for redox-sensitive structural changes in macromolecular/supramolecular assemblies.[1d, 1e] The liposomes composed of phospholipid with trimethyl-locked quinone as a part of the hydrophilic head group have entrapped a hydrophilic fluorescent dye, calcein, and release the dye upon a chemical reduction by sodium dithionite.[1e] Bio-imaging probes based on bioreductive cleavage of trimethyl-lock quinone by reductive enzymes have also been reported for potential application for tumor imaging based on redox changes occurring in tumor hypoxia.[2] To our best knowledge, thus far, there was no report for drug delivery studies with redox-sensitive polymeric carriers based on the trimethyl-locked benzoquinone group.

Benzoquinone with sterically restricted trimethyl-lock group was modified to 2-aminoethyl methacrylate for its application for radical polymerization. The monomer **3** which contains the building blocks of benzoquinone moiety and methacrylate unit was synthesized with carboxylic acid **1a** or succinimidyl ester **1b** and 2- aminoethyl methacrylate **2** by dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), and 1-hydroxybenzotriazole (HOBt).



1a (R = H)
1b (R=succinimidyl ester)

2

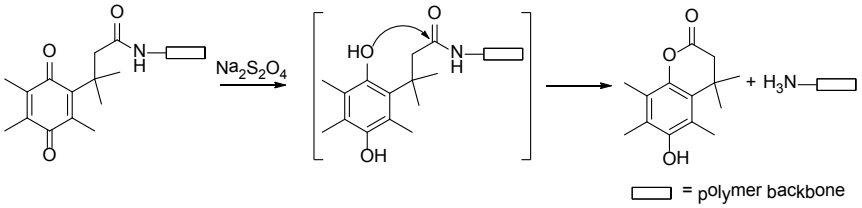
3

Scheme 1. Synthetic reaction scheme to prepare redox-sensitive monomer for radical polymerization

To a solution of pre-synthesized carboxylic acid (250mg, 1mmol) or succinimidyl ester (347mg, 1mmol) **1a** or **1b** and 2-aminoethyl methacrylate **2** (166mg, 1mmol) in dry dichloromethane (15 mL), HOBt (139mg, 1mmol), triethyl amine (101mg, 1mmol), DCC (206mg, 1mmol), and DMAP (24mg, 0.2mmol) were consecutively added at 0°C under nitrogen atmosphere. The mixture was stirred for 12 hours at room temperature with protecting light. For work-up process, the reaction mixture was cooled down with ice-bath and the formed urea byproduct was filtered off. The filtrate was washed with dichloromethane, saturated sodium bicarbonate solution, and water. The mixture was dried over with Na_2SO_4, filtered, and concentrated under reduced pressure. The monomer **3** was purified by silica gel chromatography with chloroform/ethyl acetate (19/1) solvent to afford the monomer **3** (231mg from **1a** and 246mg from **1b**) as yellow crystalline. The synthesized monomer was tested for radical polymerization to prepare a redox-sensitive polymer. However, the monomer could not be polymerized. Thus, biodegradable redox-sensitive polymers were designed as an alternative. In addition, cyclized RGD peptide was also successfully attached to the polymer ends.

Biodegradable redox-sensitive polymer and its nanoparticle[3]

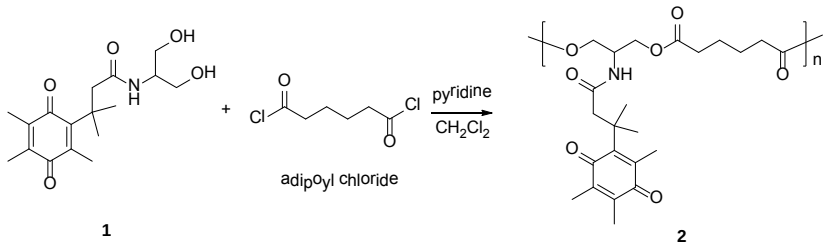
We designed a novel redox-sensitive polymer containing amino groups protected with trimethyl-locked quinone, which is able to respond to the redox variation with polymer property changes. The polymer is designed to release a lactone from trimethyl benzoquinone and unmask free amino groups via the two-electron reduction mediated by chemical agents such as sodium dithionite ($Na_2S_2O_4$) and sodium borohydride ($NaBH_4$). As a result, the reduced polymer would have enhanced water solubility at neutral pH upon protonation of free amino groups. Thus, nanoparticles prepared with the redox-sensitive polymer would be able to release incorporated drugs upon polymer hydration in response to redox changes (Scheme 1).[4]



Scheme 2 Chemical reduction of the redox-sensitive polymers based on trimethyl-locked benzoquinone.

Redox-sensitive polymer was designed from the monomer with trimethyl-lock quinone. Initially, benzoquinone carboxylic acid (*β*,*β*,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid) activated with N-hydroxylsuccimide was synthesized according to the previous reports.[1a] The activated compound was coupled with serinol (2-amino-1,3-propanediol) as a redox-sensitive diol monomer to yield serinol-derived polyester upon esterification with diacyl chloride. Serinol was selected for polymerization because of proven biocompatibility of the serinol-derived polyesters.[5] A coupling reaction between trimethyl-lock benzoquinone succinimidyl ester and serinol in a basic condition, successfully yielded diol monomer containing benzoquinone (compound **1**), which was confirmed by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis.

Polymerization was performed with synthesized the serinol monomer and adi poyl chloride under a basic condition in dichloromethane at room temperature (Scheme 1). The isolated yield of the polymerization was 85% after purification. The molecular weight and PDI were determined to be 9800Da and 1.51, respectively, by gel permeation chromatography (GPC) with polystyrene standards.



Scheme 3 Synthesis of redox-sensitive polymer with adipoyl chloride under basic condition

The proton NMR spectrum of polymer **2** in $CDCl_3$ showed all the characteristic peaks and splitting. The chemical shift of 4 protons, which are located in adjacent carbonyl groups in adipoyl groups appeared at δ = 2.33 ppm and the trimethyl group in benzoquinone ring and dimethyl groups in the serinol monomer were shown at δ = 2.12, 1.95, and 1.41 ppm, respectively. Compared to the peak shapes in monomers, corresponding peaks in the polymer were also broadened after the polymerization as expected.

Preparation and characterization of multifunctional nanoparticles[3]

Redox-sensitive polymeric nanoparticles were prepared from the synthesized polymer by an emulsion method.[6] After dissolving the synthesized polymer in a small amount of dichloromethane, an oil-in-water emulsion was made by adding the polymer solution into the aqueous solution containing tween 80 as a surfactant. The prepared polymeric nanoparticles was isolated by centrifugation, purified by rinsing with ionized water and lyophilized to yield a yellowish fluffy powder. Characteristics of polymeric nanoparticles can also be controlled with the phase volume ratio in the emulsion, surfactant, and molecular weight of the synthesized polymers.

The nanoparticles were characterized by dynamic light scattering (DLS) and transmission electron

microscopy (TEM). Particle size determined by dynamic light scattering (DLS) using a Zetasizer (Malvern Instruments, UK) confirmed successful nanoparticle preparation with a Z-averaged diameter of 180nm (PDI = 1.138) (Figure 1). A TEM image showed that the morphology of the nanoparticles was spherical and the size of particles was comparable to that determined by DSL (Figure 1).

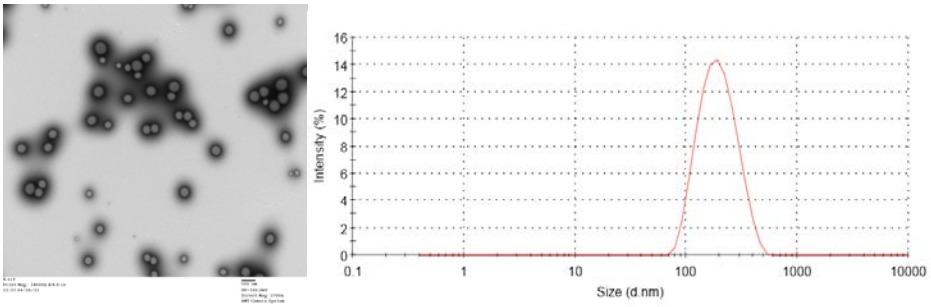


Figure 1 TEM image and size distribution of polymeric nanoparticles by dynamic scattering method (DLS)

We investigated the physical property change in the nanoparticles upon an exposure to sodium dithionite ($Na_2S_2O_4$). It was expected that sodium dithionite would reduce trimethyl-lock benzoquinone to leave free amine groups on the polymer, which would result in nanoparticles swelling or dissolution at pH 7.4. Size distribution of the nanoparticles was changed by the reducing reagent as expected. The diameter of the nanoparticles dramatically increased from 178 nm to a size which is beyond the limit of the Zetasizer as shown in Figure 2. The size changes might be induced by the protonation of free amine groups.

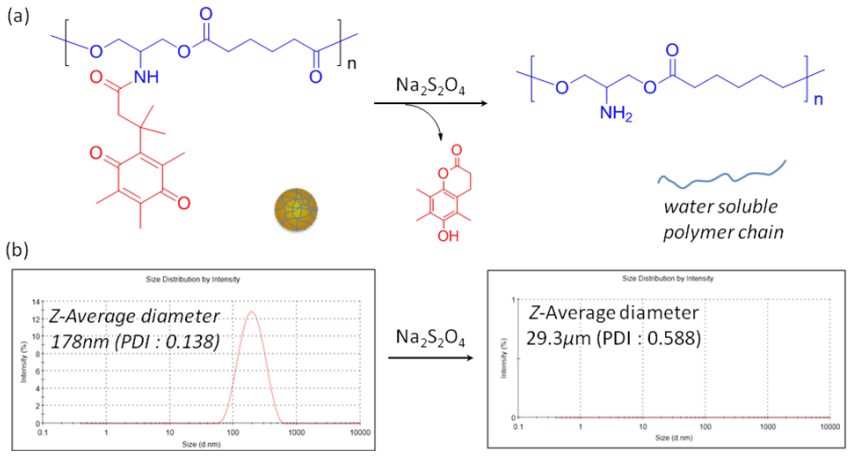


Figure 2 Change in nanoparticle size in the presence of sodium dithionite determined by DLS

A hydrophobic anticancer drug, paclitaxel, was loaded into the redox-sensitive polymeric nanoparticles. The size of paclitaxel-incorporated nanoparticles was determined to be 267 nm (PDI = 0.21) by DLS, which was slightly greater than that of blank nanoparticles. The size increase upon paclitaxel incorporation might be at tributed to the hydrophobicity of paclitaxel.[7] As reported with amphiphilic polymer micelles, hydrophobic interaction between paclitaxel and redox-sensitive polymer could expand the particles. However, the morphology of drug-loaded nanoparticles observed by TEM was similar to that of blank nanoparticles. Paclitaxel loading efficiency in the nanoparticles was determined to be 77.9% when a drug to polymer ratio of 1:10 was used.

Redox-triggered drug release from the nanoparticles was tested by in vitro release study using a medium containing sodium dithionite. At a 200 molar equivalent of sodium dithionite to the molar amount of benzoquinone, the release of paclitaxel and lactone was dramatically increased over 36 hr as shown in Figure 3. The inclusion of sodium salicylate at a concentration of 0.8 M maintained sink conditions during the release study. It has been known that sodium salicylate is able to increase paclitaxel solubility in aqueous solution without affecting physical properties of paclitaxel.[8] As shown in Figure 3, about 52% of encapsulated paclitaxel was released within 3 hours in the presence of sodium dithionite while 13% of paclitaxel was released over 12 hours in the absence of the reducing agent. An achieved cumulative paclitaxel release over 36 hr was 79% in the presence of the reducing agent. However, only 17% of

incorporated paclitaxel was released from the redox-sensitive nanoparticles without the chemical reductant.

Lactone release upon nanoparticle reduction indicated that property change occurred in the redox-sensitive nanoparticles was indeed mediated by two-electron reduction of benzoquinone moiety. Lactone release from the paclitaxel-loaded nanoparticles was also comparable to the drug release. On the other hand, lactone was not release at all in the absence of sodium dithionite as expected. Cumulative lactone release lasted for 12 hr to reach 66.8% (Figure 3 (b)).

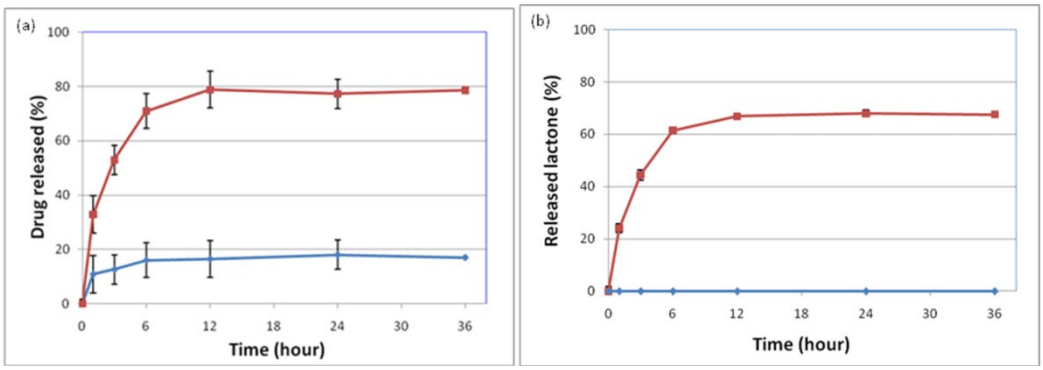


Figure 3 In vitro release profiles of (a) paclitaxel and (b) lactone in the presence of sodium dithionite (red line) and without sodium dithionite (blue line) in PBS. Significant increase in cumulative paclitaxel release was noted during the release study. Data represent as the mean ± SD (*n*=3).

We also challenged the nanoparticles with sodium dithionite after incubating them in PBS for 48 hr. An addition of the reducing agent immediately facilitated drug release which was culminated within 24 hr. Percent release of paclitaxel for 48 hr in PBS was only 20%. However, paclitaxel release was facilitated by sodium dithionite and reached to a cumulated release of 90% within 24 hr. These results indicated that the paclitaxel could be released from the trimethyl-lock quinone-based redox-sensitive polymer nanoparticles in response to two-electron reduction. Considering that enzymes catalyzing two-electron reduction are overexpressed in most cancer, the nanoparticles would provide an effective means to target solid tumors under hypoxia.

Drug release from the redox-sensitive nanoparticles is currently undergone with DT-diaphorase and NADPH. DT-diaphorase-triggered lactone release from the synthesized monomer was confirmed by the measurements of released lactone with HPLC.

In vitro evaluation of nanoparticles on cultured MDA-MB-231 breast cancer cells

Currently paclitaxel-incorporating redox-responsive nanoparticles have been improved for colloidal stability in water. After preliminary experiments using mannitol, dispersible nanoparticles with paclitaxel could be successfully prepared by freeze drying. In vitro efficacy of the nanoparticle formulation towards MDA-MB-231 will be tested under hypoxic and normoxic conditions.

Key Research Accomplishments

-Synthesis and characterization of a redox-sensitive monomer based on trimethyl-lock benzoquinone
-Synthesis and characterization of a redox-sensitive biodegradable polyester
-Fabrication and characterization of redox-sensitive nanoparticles that incorporated paclitaxel
-Redox-sensitive paclitaxel release from the synthesized multifunctional nanoparticles
-Stabilization of the nanoparticle formulation by freeze drying

Reportable Outcomes

Publications

1. Cho, H.; Bae,J.; Garripelli,V.K.; Anderson,J.; Jun,H.-W.; Jo,S., Redox-sensitive polymeric nanoparticles for drug delivery, *Chem. Coummun.*, **48**, 6043-6045. (2012).
2. Cho, H.; Jo S., UM Provisional Patent Application, pending (2012).

Conference Poster Presentations

1. Bae, J; Nael, M.A.; Doerksen, R.J.; Jo, S., Trimethyl-locked benzoquinone based polymeric nanoparticles for targeted drug delivery, the American Association of Pharmaceutical Scientist (AAPS) Annual Meeting and Exposition, Chicago, IL, scheduled in October, 2012.

2. Bae, J; Nael, M.A.; Anderson, J.M.; Doerksen, R.J.; Jun, H-W; Jo, S., Novel redox-sensitive polymeric nanoparticles for targeted delivery of anti-cancer agents, 5th Annual Mississippi Biophysical Consortium Annual Meeting, Hattiesburg, MS (2012).
3. Bae, J; Nael, M.A.; Doerksen, R.J.; Jo, S., Redox-sensitive polymeric nanoparticles for targeted cancer drug delivery**,** MS EPSCoR Annual Meeting, Oxford, MS (2012).
4. Cho, H. and Jo, S., Redox-sensitive polymeric nanoparticles, 242nd American Chemical Society (ACS) National Meeting & Exposition, Denver, CO (2011).
5. Jo, S. and Cho, H., Hypoxia-sensitive multifunctional nanoparticles for targeted drug delivery to breast cancer, 2011 Era of Hope Conference in Orlando, FL, August 2-5, 2011.

Invited Seminar Presentations

1. Jo, S., Bioresponsive drug delivery using tailored synthetic materials, Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, TN, January 30, 2012.
2. Jo, S., Hypoxia Targeting Revisited for Cancer Drug Delivery, The 6th International Symposium on Intelligent Drug Delivery System, Seoul, Korea, March 14-17, 2012.

Funding Applied

1. Mississippi State University/National Science Foundation EPS-0903787 09/2011 – 08/2012
   Title: Computational modeling-aided design, synthesis and evaluation of redox-sensitive polymer nanoparticles for cancer targeted drug delivery, $54,504 (awarded)
   Role**:** Principal Investigator
2. Mississippi State University/National Science Foundation EPS-0903787 09/2012 – 08/2013
   Title: Computational modeling-aided design, synthesis and optimization of redox-sensitive polymer nanoparticles with optimal colloid-forming and DT-diaphorase-substrate properties, $66,283 (awarded)
   Role**:** Principal Investigator

Employment and research opportunities

1. One post-doctoral research scientist, Dr. Hanjoung Cho, had been employed from Oct. 2010 to Sep. 2011.
2. Two graduate students have experienced research about redox-sensitive polymer synthesis.

Conclusion

Novel redox-sensitive polymeric nanoparticles were prepared by an emulsion method from a synthesized monomer containing trimethyl-lock quinone as a redox sensitive group. A hydrophobic cancer drug, paclitaxel, was incorporated in the polymeric nanoparticles and was released by reduction in a triggered manner. Since the novel polymeric nanoparticles are able to release incorporated drugs in response to a redox status, the polymeric nanoparticles would be suitable for targeted drug delivery to hypoxic solid breast cancer.

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Appendices

Abstracts

Bae, J; Nael, M.A.; Doerksen, R.J.; Jo, S., Trimethyl-locked benzoquinone based polymeric nanoparticles for targeted drug delivery, the American Association of Pharmaceutical Scientist (AAPS) Annual Meeting and Exposition, Chicago, IL, scheduled in October, 2012.

Trimethyl-locked benzoquinone based polymeric nanoparticles for targeted drug delivery

J. Bae, M. Nael, R. Doerksen, S. Jo
The University of Mississippi

Purpose

The objective of this study was to develop redox-sensitive polymeric nanoparticles encapsulating drugs for tumor specific
delivery, based on computational modeling.

Methods

The polymerization was performed in basic conditions with the selected monomer with a trimethyl-locked benzoquinone
moiety based on the calculation of logP (lipophilicity) for several proposed structures using ChemAxon software. The synthesized redox-sensitive polymers with succinyl chloride, glutaryl chloride, and adipoyl chloride were characterized by proton NMR spectroscopy and gel permeation chromatography (GPC). Bioresponsive polymeric nanoparticles were prepared from the synthesized polymers with an anticancer drug, paclitaxel, by an emulsion method. Its z-average sizes and drug loading efficiency in the nanoparticles were determined by dynamic light scattering using a Zetasizer (Malvern Instruments, UK) and an HPLC method, respectively. The dimensions of the redox-sensitive nanoparticle were simulated by packmol. In vitro redox-triggered drug release was tested using a chemical reductant, sodium dithionite, a reductive enzyme, DT- diaphorase, and NADPH.

Results

LogP-based computational modeling guided the selection of monomers with rational prediction of polymer properties.
Synthesized novel redox-sensitive polymers were characterized by NMR spectroscopy. The molecular weights of redox- sensitive polymers from succinyl chloride, glutaryl chloride, and adipoyl chloride were affected by synthetic conditions and ranged from 2000 to 6000 Da/mol. Nanoparticle size also varied with polymers and was determined to be smaller than 300 nm. In vitro drug release from the redox-sensitive nanoparticles was triggered by the reduction of polymer either with sodium dithionite or with DT-diaphorase/NADPH. The nanoparticles were able to release incorporated paclitaxel in response to the redox capacity in the release media.

Conclusion

Novel redox-sensitive polymers were synthesized with an application of bioreductive trimethyl-locked benzoquinone with an
aid of computational modeling. Nanoparticles were prepared from the synthesized polymers for drug delivery applications. The redox-sensitive polymeric nanoparticles would be useful for tumor targeted drug delivery with favorable size distribution for enhanced permeability and retention (EPR) and redox-triggered drug release from the nanoparticles.

Bae, J; Nael, M.A.; Anderson, J.M.; Doerksen, R.J.; Jun, H-W; Jo, S., Novel redox-sensitive polymeric nanoparticles for targeted delivery of anti-cancer agents, 5th Annual Mississippi Biophysical Consortium Annual Meeting, Hattiesburg, MS (2012).

Novel redox-sensitive polymeric nanoparticles for targeted delivery of anti-cancer agents

JungeunBae, Manal A. Nael, Joel M. Anderson, Robert J. Doerksen, Ho-Wook Jun, Seongbong Jo

Novel redox-sensitive polymeric nanoparticles for targeted delivery of anti-cancer agents
JungeunBae, Manal A. Nael, Joel M. Anderson, Robert J. Doerksen, Ho-Wook Jun, Seongbong Jo

The application of bioresponsive polymeric particles in the scale of nanometers has drawn immense interest as a controlled delivery system for anticancer drugs. Trimethyl-locked benzoquinone group has been used to trigger the release of cytotoxic drugs within cancer microenvironments via redox-induced intra-molecular cyclization to form a lactone. We designed novel redox-sensitive polymers with trimethyl-locked benzoquinone chemistry with an aid of computational modeling. Polymer structures built from monomer combinations were evaluated for the prediction of polymer property changes upon r eduction. Two polymer structures based on t rimethyl-locked benzoquinone modified serinol and dicarboxylic acids, adipoic acid and glutaric acid, were selected and synthesized. Polymeric nanoparticles were prepared from the synthesized polymers. Redox-triggered release of an anticancer drug, paclitaxel, was demonstrated from the nanoparticles. Here, we report an important application of computational modeling for the development of polymeric biomaterial which can be useful for drug delivery.

Cho, H. and Jo S., Redox-sensitive polymeric nanoparticles, 242nd American Chemical Society (ACS) National Meeting & Exposition, Denver, CO (2011).

Redox-sensitive polymeric nanoparticles

The polymeric nanoparticles have been extensively pursued for the development of targeted-drug delivery systems. Herein, we present polymeric nanoparticles containing redox-sensitive moieties, which can be removed by chemically or enzymatically triggered reduction. Since the trimethyl lock-based functional groups undergo the triggered reduction to release a lactone and expose free hydrophilic functional groups, a class of trimethyl lock chemistry-based monomers were synthesized and polymerized to obtain biodegradable polymers for the redox-sensitive nanoparticles. The alteration of physicochemical properties of the polymer induced by the triggered reduction can dissemble the nanoparticles, which facilitates the release of entrapped chemicals. Nanoparticles were prepared from the redox-sensitive biodegradable polymers via an emulsion method. The dynamic light scattering ensured that the size of nanoparticle significantly changed upon chemical reduction with sodium hydrosulfite. In addition, the nanoparticles were challenged to a reductive enzyme frequently overexpressed in cancer, DT-diaphorase, to release the lactone and the amounts of released lactone were quantitatively determined by HPLC. The nanoparticles that are able to response to the triggered reduction would be promising as a drug delivery platform to target tumors.

Redox-sensitive polymeric nanoparticles for targeted cancer drug delivery

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Department of Pharmaceutics[1], Department of Medicinal Chemistry[2],
Research Institute of Pharmaceutical Sciences[3], School of Pharmacy, The University of Mississippi, MS, USA
Research Focus Area: Computational Chemistry
EPSCoP participation: 9/1/2011-Present

Introduction

Nanoscale drug delivery systems offer effective methodologies to target tumor microenvironments via adaptation of pathophysiological changes occurring in tumors. Redox-stress developed in hypoxic cancer cells and overexpression of reductive enzymes in cancer cells have provided a c ompelling rationale to take advantage of redox-sensitive materials in cancer research. It has also been noted that various nanoparticles could be preferentially accumulated in solid tumors by the enhanced permeability and retention (EPR) through abnormal tumor vasculatures [1]. We proposed a polymer nanoparticle-based drug delivery system that can enhance the targeting efficiency towards tumors with the redox-sensitivity and preferable particle distribution in tumors. The trimethyl-locked benzoquinone-based redox-sensitive polymers were rationally designed via computational modeling. Nanoparticles were prepared from the synthesized polymers and tested for *in vitro* drug release [2].

Significance

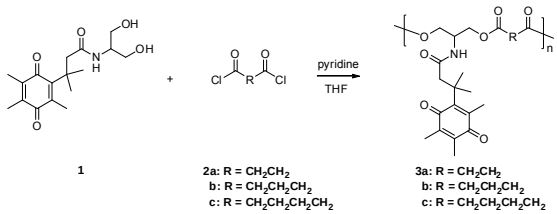
Computational modeling-based polymer design allows rational prediction of physicochemical properties of the redox polymers and their nanoparticles after/before polymer reduction, which can be correlated to the patterns of drug release. The method would be a facile and cost-effective approach to develop novel functional biomaterials. In addition, redox-sensitive nanoparticles would provide an effective means to deliver cytotoxic cancer drugs selectively to the affected disease sites to enhance therapeutic effects while minimizing dose-limiting systemic toxicities.

Research Summary and Status

A battery of redox-sensitive polymers were synthesized and used for the preparation of redox-sensitive nanoparticles. Computational modeling and simulation have been applied for the prediction of physico-chemical property changes occurring in the synthesized redox-sensitive nanoparticles, which would be translated into redox-sensitive drug release.

Aim 1: Computational modeling-based design of redox-sensitive biodegradable polymers and their synthesis.

Results: A group of monomers were examined. Polymers constructed from the combination of selected monomers were theoretically tested for changes in their key physical property, log P. The polymers with a redox-sensitive trimethyl-locked benoquinone moiety were synthesized from the selected monomers (Scheme 1).



Scheme 1.Reaction scheme for polymer synthesis

Synthesized polymers were characterized by proton NMR spectroscopy and gel permeation chromatography (GPC). The molecular weights of 3a, 3b, and 3c were found to be 2000 Da, 2500 Da and 4000 Da respectively.

Aim 2: Redox-sensitive nanoparticle preparation with the synthesized polymers.

Table 1 logP values of synthesized polymers

Polymer (MW ~5000)	Non reduced	Partially reduced	Fully reduced
3a	22.10	5.56	-10.49
3b	27.81	10.30	-6.29
3c	32.46	16.27	-0.55

***Results*:** Redox-sensitive nanoparticles were obtained with the synthesized polymers 3a, 3b, and 3c by a single emulsion method. Z-average sizes of the nanoparticles prepared from 3a, 3b, and 3c were determined to be 91.63 nm, 68.70 nm, and 118.00 nm, respectively with a Zetasizer (Malvern instruments, UK)

Aim 3: *In vitro* drug release from the redox-sensitive nanoparticles under simulated redox-stress either with chemical or enzymatic reducing components.

Results: An anticancer drug, paclitaxel, was incorporated into the redox-sensitive nanoparticles. *In vitro* drug release is currently underway with DT-diaphorase in the presence of NADPH. The dimensions of the redox-sensitive nanoparticle were simulated by computational modeling (Figure 1).

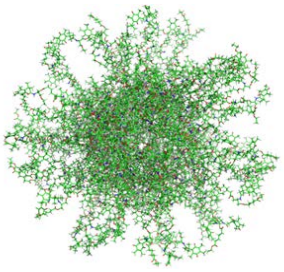


Figure 1 Spherical polymer particle constructed by packmol

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Jo, S. and Cho, H., Hypoxia-sensitive multifunctional nanoparticles for targeted drug delivery to breast cancer, 2011 Era of Hope Conference in Orlando, FL, August 2-5, 2011.

Hypoxia-sensitive multifunctional nanoparticles for targeted drug delivery to breast cancer

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Hypoxia in breast cancer has implicated tumor progression by adaptive responses of tumor cells including enhanced invasion/migration of endothelial cells and therapy resistance, which results in poor prognosis and patient outcomes. Cyclized RGD peptides selectively bind to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins overexpressed on the surface of angiogenic endothelial cells in breast cancer. Thus, dual-targeting nanoparticles that preferably accumulate in tumor via the EPR effect, effectively bind to angiogenic endothelial cells in tumor neovasculatures, and selectively release cytotoxic drugs in hypoxic cancer cells to achieve a high drug concentration would synergistically inhibit breast cancer while minimally affecting normal cells.

Multi-functional nanoparticles intended for dual-targeting of hypoxia and angiogenesis have been sought for highly selective drug delivery to breast cancer. Herein, we present polymeric nanoparticles containing redox-sensitive moieties which can be shed by chemically or enzymatically triggered reduction. Since the trimethyl lock-based functional groups undergo the triggered reduction to release a lactone and expose free hydrophilic functional groups, a class of trimethyl lock chemistry-based monomers were synthesized and polymerized to obtain redox-sensitive nanoparticles. Various monomers subjected to radical or step polymerization have been synthesized to incorporate trimethyl lock-leaving groups on polymer backbones. Nanoparticles were prepared either by continuous stirring of an o/w emulsion containing a redox-sensitive biodegradable polymer in oil phase or by radical polymerization of a vinyl monomer carrying a trimethyl lock leaving group in the presence of a divinyl crosslinking agent. The average size of the prepared nanoparticles was determined to be around 200 nm by the dynamic light scattering (DLS). Chemical reduction with sodium hydrosulfite resulted in the disappearance of the particle size corresponding to the nanoparticles on the size distribution, which indicates the dissembling of the nanoparticles. In addition, the nanoparticles were challenged to a reductive enzyme frequently overexpressed in cancer, DT-diaphorase, to release the lactone and released lactone was quantitatively determined by HPLC. An anticancer drug, paclitaxel, will be incorporated in the nanoparticles and cyclic RGD peptide will be conjugated to the nanoparticles. The multifunctional nanoparticles containing paclitaxel will be tested on cultured breast cancer cells for in vitro efficacy.

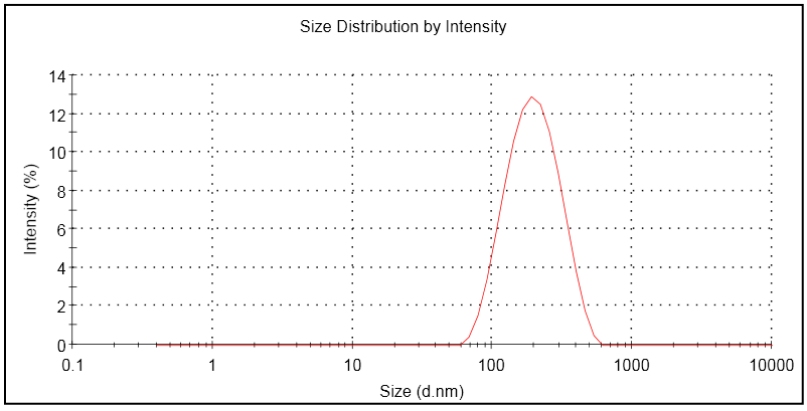

Figure 1. Size distribution of the redox-sensitive nanoparticles determined by DSL

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COMMUNICATION

Redox-sensitive polymeric nanoparticles for drug delivery†

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Bioresponsive polymeric nanoparticles have been extensively pursued for the development of tumor-targeted drug delivery. A novel redox-sensitive biodegradable polymer with "trimethyl-locked" benzoquinone was synthesized for the preparation of paclitaxel-incorporated nanoparticles. The synthesized redox-sensitive nanoparticles released paclitaxel in response to chemically triggered reduction.

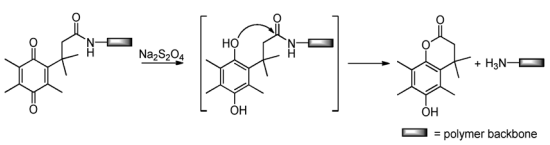
Multifunctional polymeric nanoparticles have been widely adopted for cancer-targeted drug delivery with their potential therapeutic benefit to minimize the dose-limiting systemic toxicity by preferential accumulation in tumors *via* the enhanced permeability and retention (EPR) effect.[1] These types of nanoparticles have frequently been modified to respond to the signals stemming from tumor microenvironments so that they can release incorporated drugs selectively in tumor sites.[2] Thus far, various bioresponsive materials that are sensitive to external stimuli, such as pH,[3] temperature,[4] enzymes,[5] and light,[6] have been intensively considered for targeted drug delivery to cancer.

Since redox changes associated with tumor hypoxia have been identified as a viable biomarker for tumor progression and cancer drug resistance, reductive enzymes overexpressed in tumor microenvironments, such as 12- to 18-fold overexpression of DT-diaphorase (EC 1.6.99.2) in lung cancer,[7] provide an important strategy for selective tumor targeting. Various bioreductive prodrugs have been reported and some of them are currently under clinical trials.[8] However, the prodrug approach to target tumors may be complicated with ubiquitous expression of various reductive enzymes in normal cells.[9]

Trimethyl-locked benzoquinone (TMBQ) is known to be chemically or enzymatically transformed into lactone *via* intramolecular cyclization triggered by two-electron reduction.[10,11] Prodrugs and bio-imaging probes have been based on the bioreductive cleavage of TMBQ by reductive enzymes and suggested for solid tumor-selective drug delivery and imaging based on redox changes occurring in tumor hypoxia.[10] As demonstrated with a bioreductive TMBQ-based aniline mustard prodrug, tested on T47D cells, the active drug resulted in cytotoxicity after the reductive activation by oxidoreductases at a low oxygen concentration.[10a] Even redox-triggered liposomes that electrochemically release chemicals have been designed with the TMBQ chemistry.[11e] Chemical reduction-induced shedding of TMBQ from the liposome surface resulted in the structural change of the supramolecular assemblies, which later destabilized the redox-sensitive liposomes to electrochemically release a hydrophilic fluorescent dye, calcein.[11e] However, the TMBQ-based redox-chemistry has never been applied for a polymeric drug delivery system thus far. The approach to use TMBQ-based redox-sensitive polymeric nanoparticles is advantageous over the TMBQ-based prodrugs or liposomes to target the redox stress in solid tumors. Polymeric nanoparticles can be easily modified with the ligands that selectively interact with the molecules expressed on cancer cells to achieve active targeting. In addition, the nanoparticles may be more flexible than the above-mentioned liposome for the formulation of hydrophobic cancer drugs since they may disrupt exquisitely balanced non-covalent interactions of TMBQ at the liposome surface. Furthermore, the nanoparticles can easily achieve prolonged circulation in blood and preferred accumulation in tumor by poly(ethylene glycol) (PEG) coupling.[12]

In this article, we describe a proof-of-concept novel redox-sensitive polymer containing amino groups protected by TMBQ. The polymer is intended to release lactone and unmask free amino groups under a simulated redox stress with sodium dithionite. Thus, its nanoparticle would be able to release incorporated drugs upon increased polymer hydration and subsequent nanoparticle swelling with the protonation of free amino groups at physiological pH (Scheme 1).[11] We primarily focused to test the feasibility of novel TMBQ-based redox-sensitive polymer nanoparticles as a bioresponsive drug delivery system using a simple *in vitro* drug release study.

A redox-sensitive polymer was designed and synthesized from a monomer containing TMBQ. Initially, benzoquinone carboxylic acid (β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid) activated with *N*-hydroxysuccimide



Scheme 1 Chemical reduction of the redox-sensitive polymers based on trimethyl-locked benzoquinone.

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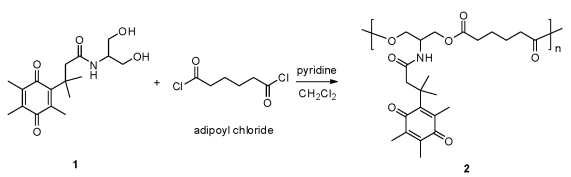
† Electronic supplementary information (ESI) available: Detailed experimental procedures and characterization data. See DOI: 10.1039/c2cc31463k

was synthesized according to the previously reported procedure.[11a] The activated compound was coupled with serinol (2-amino-1,3-propanediol) to yield a redox-sensitive diol monomer (ESI†). Serinol was selected for the polymer synthesis because of its proven biocompatibility as a monomer for biodegradable polyesters.[13] A coupling reaction between TMBQ-succinimidyl ester and serinol consequently yielded a diol with a pendant TMBQ (compound **1**). Synthesized serinol monomer was successfully confirmed by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis. The serinol monomer was polymerized by the reaction described in Scheme 2. The isolated yield of the synthesized redox-sensitive polyester was found to be 85% after purification with precipitation in ether. The number-average polymer molecular weight (M_n) and PDI were determined to be 9800 Da and 1.51, respectively, by gel permeation chromatography (GPC) with polystyrene standards.

The proton NMR of polymer **2** in $CDCl_3$ showed all the characteristic peaks and splitting (ESI†), which indicated successful polymer synthesis. The chemical shift of 4 protons, which are located in adjacent carbonyls in adipoyl groups appeared at δ = 2.33 ppm indicating polyester backbone formation. Additionally, the trimethyl group in benzoquinone ring and dimethyl groups in the serinol monomer were shown at δ = 2.12, 1.95, and 1.41 ppm, respectively. The proton peaks of the monomer were broadened after the polymerization. It is worthwhile to note that this synthetic reaction is also amenable to further PEG modification at the redox-sensitive polymer chain ends by the treatment of adipoyl chloride to secure terminal carboxyl groups that can be exploited for the esterification with methoxy-capped PEG. Even cancer selective cyclic RGD can be modified to the end carboxyl groups.

Redox-sensitive polymeric nanoparticles were prepared from the synthesized polymer by an emulsion method.[14] Tween 80 was used as a surfactant to form emulsion instead of poly(vinyl alcohol) partially because of a low polymer molecular weight of the redox polymer. The final polymeric nanoparticles resulted in a yellowish fluffy powder. Particle size determined by dynamic light scattering (DLS) using a Zetasizer (Malvern Instruments, UK) revealed successful nanoparticle preparation with a Z-averaged hydrodynamic diameter of 180 nm (PDI = 0.138) (Fig. 1) which would allow tumor site accumulation by EPR.[1] A TEM image of the nanoparticles showed that the morphology of the nanoparticles was spherical with the particle size qualitatively comparable to that determined by DLS (Fig. 1), indicating that the nanoparticle preparation *via* an emulsion was reliable.

The effect of redox stress on the nanoparticles was tested with a chemical reductant, sodium dithionite ($Na_2S_2O_4$). It was expected that sodium dithionite would reduce TMBQ to leave free amine groups on the polymer, which would result in physico-chemical changes in nanoparticles that would induce



Scheme 2 Synthesis of redox-sensitive polymer with adipoyl chloride under basic conditions.

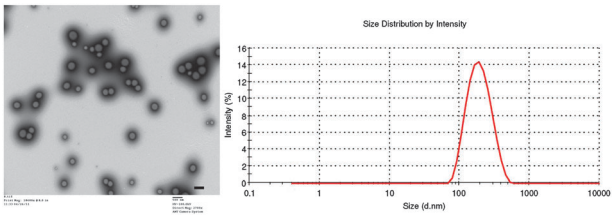


Fig. 1 TEM image and size distribution of polymeric nanoparticles by a dynamic light scattering method (DLS). Scale bar is 500 nm in length.

nanoparticle swelling or dissolution at pH 7.4. Sodium dithionite-induced reduction dramatically increased the hydrodynamic diameter of the nanoparticles from 178 nm in a turbid solution to 29.3 μm in a transparent solution, a size beyond the measurable limit by the Zetasizer as shown in Fig. 2. The size changes might have resulted from the protonation of free amine groups in serinol of which pK_a is around 9.15.

A hydrophobic anticancer drug, paclitaxel, was loaded into the redox-sensitive polymeric nanoparticles of which the particle size was determined to be 267 nm (PDI = 0.21). The slight size increase from 180 nm to 267 nm upon paclitaxel incorporation might be attributed to the hydrophobicity of paclitaxel.[15] As reported with amphiphilic polymer micelles, hydrophobic interaction between paclitaxel and redox-sensitive polymer could expand the particles.[15] However, the morphology of drug-loaded nanoparticles observed by TEM was similar to that of blank nanoparticles and spherical (ESI†). The paclitaxel loading efficiency in the nanoparticles was determined to be 77.9% when a drug to polymer ratio of 1 : 10 was used.

Redox-triggered drug release from the nanoparticles was tested by an *in vitro* release study using a medium containing sodium dithionite. At a 200 molar excess of sodium dithionite to the molar amount of benzoquinone, the release of paclitaxel and lactone was dramatically increased over 36 h as shown in Fig. 3. The amount of sodium dithionite used in the experiment might exceed the redox stress occurring in tumors. The primary purpose of using an excess amount of sodium dithionite was to test the feasibility of the nanoparticles as a novel redox-sensitive drug delivery system. Further investigation with DT-diaphorase and NADPH mimicking tumor microenvironments would better evaluate the TMBQ-based polymer nanoparticles.

The inclusion of sodium salicylate at a concentration of 80 mM maintained sink conditions during the release study. It has been known that sodium salicylate is able to increase paclitaxel

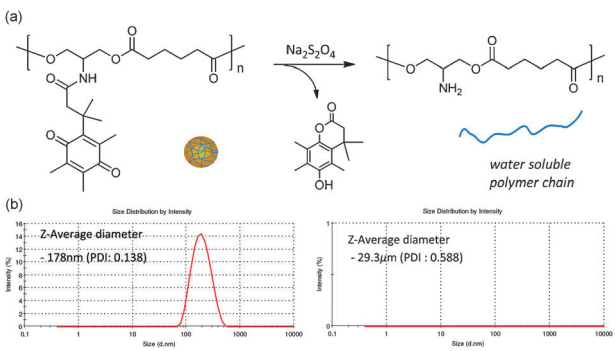


Fig. 2 Change in nanoparticle size in the presence of sodium dithionite determined by DLS.

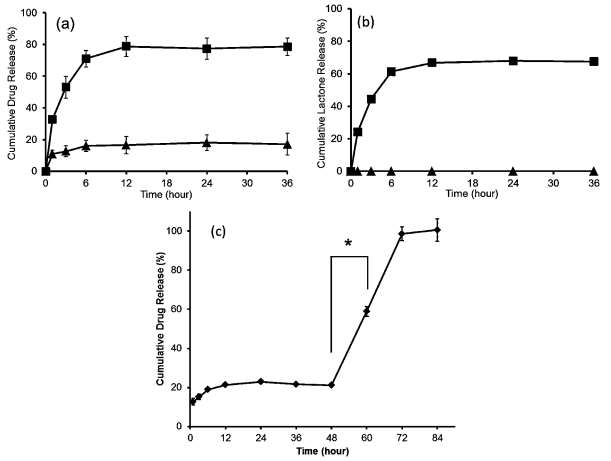


Fig. 3 *In vitro* release profiles of (a) paclitaxel and (b) lactone in the presence of sodium dithionite (■) and without sodium dithionite (▲) in PBS. Triggered paclitaxel release was tested with an alternating addition of sodium dithionite (c). Significant increase (*) in cumulative paclitaxel release ($P < 0.05$) was noted during the release study. Data represent the mean ± SD ($n = 3$).

solubility in aqueous solution without affecting physical properties of paclitaxel.[16] As shown in Fig. 3, about 52% of encapsulated paclitaxel was released within 3 h in the presence of sodium dithionite while 13% of paclitaxel was released over 12 h in the absence of the reducing agent. An achieved cumulative paclitaxel release over 36 h was 79% in the presence of the reducing agent. However, only 17% of incorporated paclitaxel was released from the redox-sensitive nanoparticles without the chemical reductant. Lactone release from the paclitaxel-loaded nanoparticles was also consistent with the paclitaxel release. Cumulative lactone release lasted for 12 h to reach 66.8% while no lactone was released without reduction (Fig. 3b). Therefore, lactone release upon nanoparticle reduction indicates that property change occurred in the redox-sensitive nanoparticles was indeed mediated by the two-electron reduction of TMBQ moiety. Furthermore, the consequent size change occurred to nanoparticles might be translated into increased paclitaxel release.

We also challenged the nanoparticles with the reducing agent after incubating them in PBS for 48 h. An addition of sodium dithionite immediately increased drug release, which peaked within 24 h (Fig. 3c). The percentage release of paclitaxel within initial 48 h was only 20%. However, paclitaxel release increased to a cumulative release of 90% within 24 h after the addition of sodium dithionite. Taken together, the results indicated that paclitaxel could be released from the TMBQ-based redox-sensitive polymer nanoparticles in response to redox changes. Although a gap needs to be filled with better simulation of the redox stress using tumor-related reductive enzymes that are able to reduce TMBQ, the TMBQ-based nanoparticles would be useful for targeting solid tumors.

Novel redox-sensitive polymeric nanoparticles were prepared from a synthesized monomer containing TMBQ as a redox sensitive group. A hydrophobic cancer drug, paclitaxel, was incorporated into the polymeric nanoparticles and released by sodium dithionite-mediated reduction in a triggered manner. Since the polymeric nanoparticles are able to release incorporated drugs in response to a simulated redox stress, these nanoparticles would be useful for targeted drug delivery to solid tumors.

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Redox-sensitive Polymeric Nanoparticles for Drug Delivery

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1. Materials

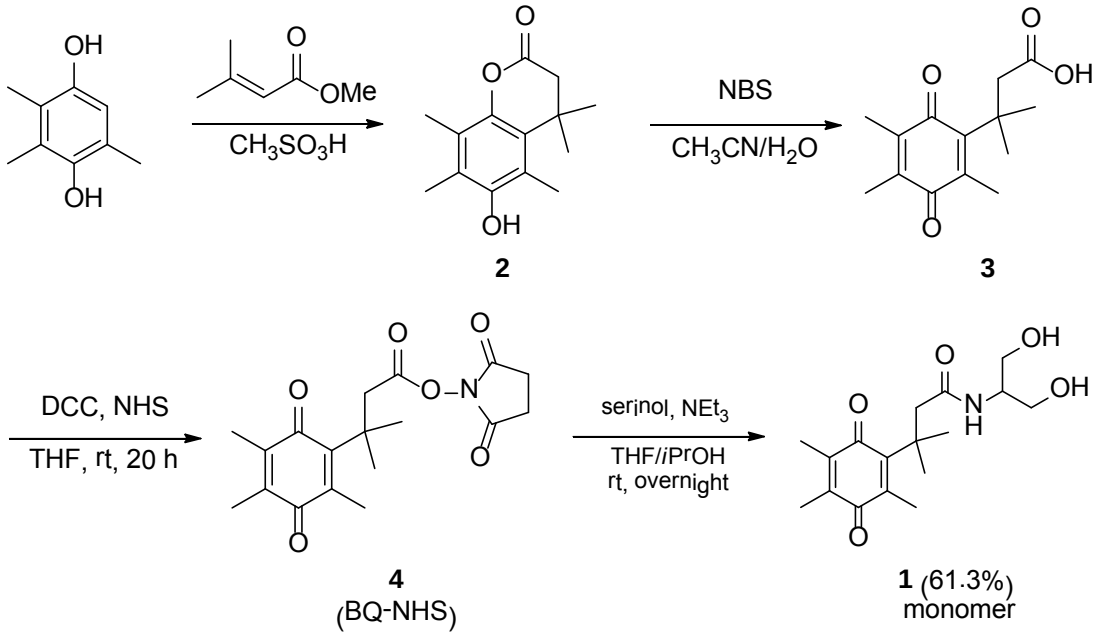
Trimethyl hydroquinone was purchased from Sigma-Aldrich (St. Louis, MO) and used as received. 2-Amino-1,3-propanediol (serinol) was purchased from Alfa Aesar (Ward Hill, MA) and used as received. All other chemicals were obtained from Fisher Scientific (Pittsburgh, PA) and used without further purification, except adipoyl chloride. Adipoyl chloride was distilled under reduced pressure right before use. Paclitaxel was purchased by LC laboratories® (Woburn, MA, USA). All solvents, unless otherwise stated, were used without further purification.

2. Synthesis of the monomer

NHS-activated benzoquinone compound **4** was synthesized, as reported previously.[9a] To a mixture of 2-amino-1,3-propanediol (1.639 g, 18 m mol) and triethyl amine (5 mL) in isopropanol (100 mL), the synthesized compound **4** (4.168 g, 12 mmol) in THF (50 mL) was added dropwise at room temperature. After being stirred overnight at room temperature, the mixture was collected and concentrated under reduced pressure. The crude compound was washed with $NaHCO_3$ three times and extracted by ethyl acetate (50 mL×3), then dried over by sodium sulfate. The reductive monomer **1** (yellow crystalline) was purified by recrystallization in ethyl acetate with an isolated yields of 61.3% (2.379 g). NMR spectra were recorded on a Bruker Ultrashield™ 400 PLUS at 400 and 100 MHz for ^1H and ^{13}C, respectively. The residual solvent peaks (δ = 2.50 or 7.24 for DMSO-d_6 or $CDCl_3$, respectively) were used as the solvent residual references for ^1H NMR spectra, and chemical shifts of the solvent peaks (δ = 39.52 or 77.00 for DMSO-d_6 or $CDCl_3$, respectively) were used as the reference for ^{13}C NMR spectra.

Monomer **1**, yellow crystal; mp 161–162 °C (from EtOAc) , ^1H NMR (400 MHz, DMSO-d_6) δ = 7.54 (d, *J* = 8.3 Hz, 1H), 4.56 (t, *J* = 5.5 Hz, 2H), 3.60 (dt, *J* = 8.2, 5.6 Hz, 1H),3.32 (m, 4H),

2.71 (s, 2H), 2.01 (s, 3H), 1.90 (s, 3H), 1.87 (s, 3H), 1.32 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 190.20, 186.85, 171.31, 154.94, 144.03, 136.07, 135.37, 60.13, 52.70, 47.58, 37.69, 28.06, 13.69, 12.77, 11.70; IR (neat, cm^{-1}) 1528, 1600, 1638, 3333; Found: C, 63.20; H, 7.71. Calcd for $C_{17}H_{25}O_5$: C, 63.14; H, 7.79.



Scheme 1. Synthetic reactions for a trimethyl-lock quinone-based redox-sensitive monomer.

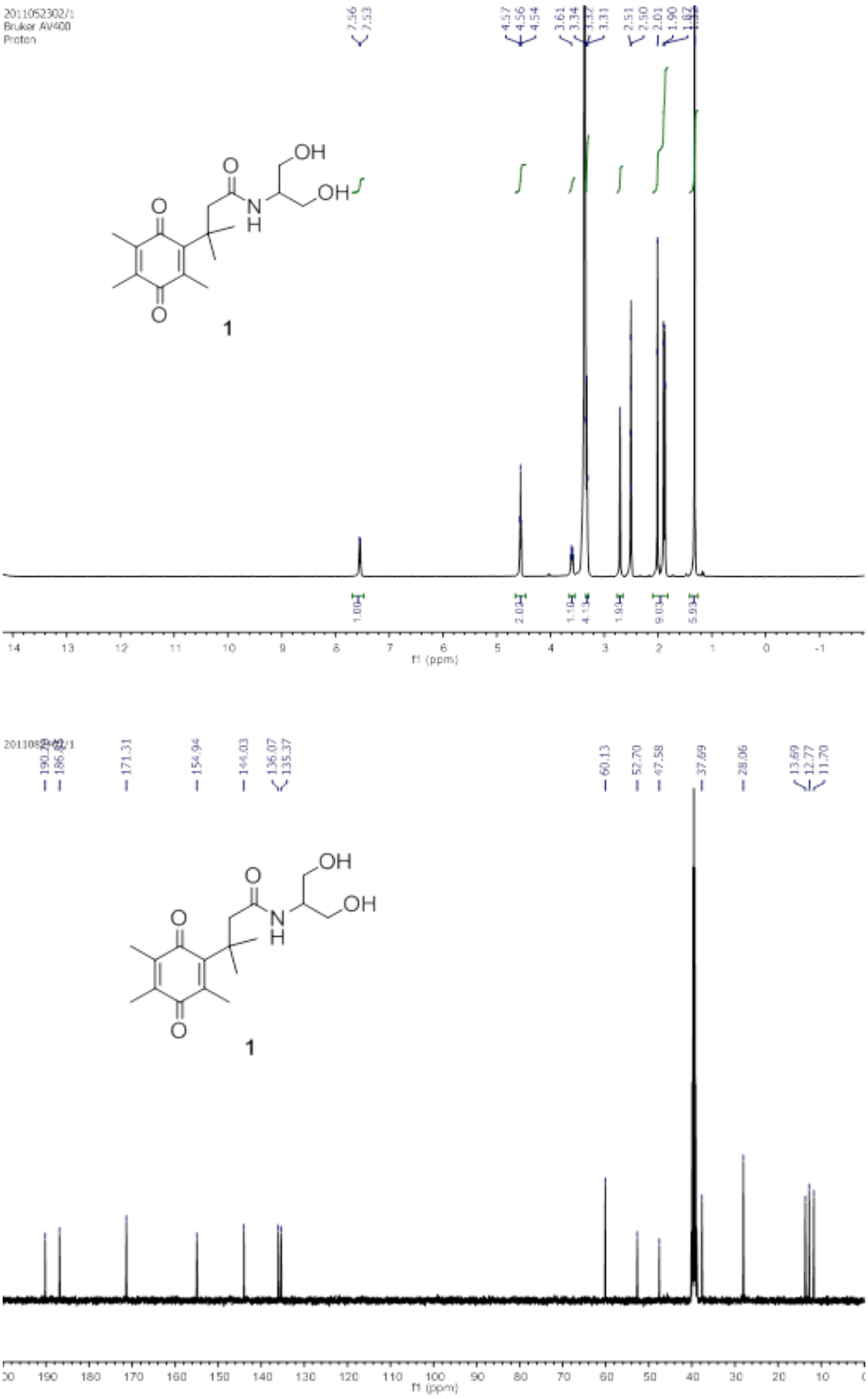


Figure 1. ^{1}H and ^{13}C NMR spectra of compound **1** in DMSO-d_6.

3. Polymerization with adipoyl chloride

To a solution of the synthesized monomer (1.3 g, 4 mmol) in pyridine (3.2 mL), adipoyl chloride (0.732 g, 4 mmol) in dichloromethane (25 mL) was added dropwise over 10 minutes at room temperature. After being stirred overnight, the mixture was collected and washed with water three times. The collected organic layer was dried with sodium sulfate and concentrated under reduced pressure. The polymer was precipitated in cold ethyl ether (20 mL), yielding yellow polymer with an isolated yield of 84.7% (1.6 g). Molecular weight and PDI of the synthesized polymer was determined to be 9800 Da (M_n) with 1.51, respectively, by GPC measurements using polystyrene standards.

A Waters gel permeation chromatography (GPC) system (Waters, Milford, MA) equipped with a binary pump (Waters 1525), a refractive index detector (Waters 2414), and a Styragel HR4E column (300 × 7.8 mm I.D., 5μm particle size) were used for the molecular weight measurements. THF of HPLC grade was eluted at a flow rate of 1mL/min at 25°C. Polystyrene standards (1,000-50,000Da) were also run to obtain a calibration curve.

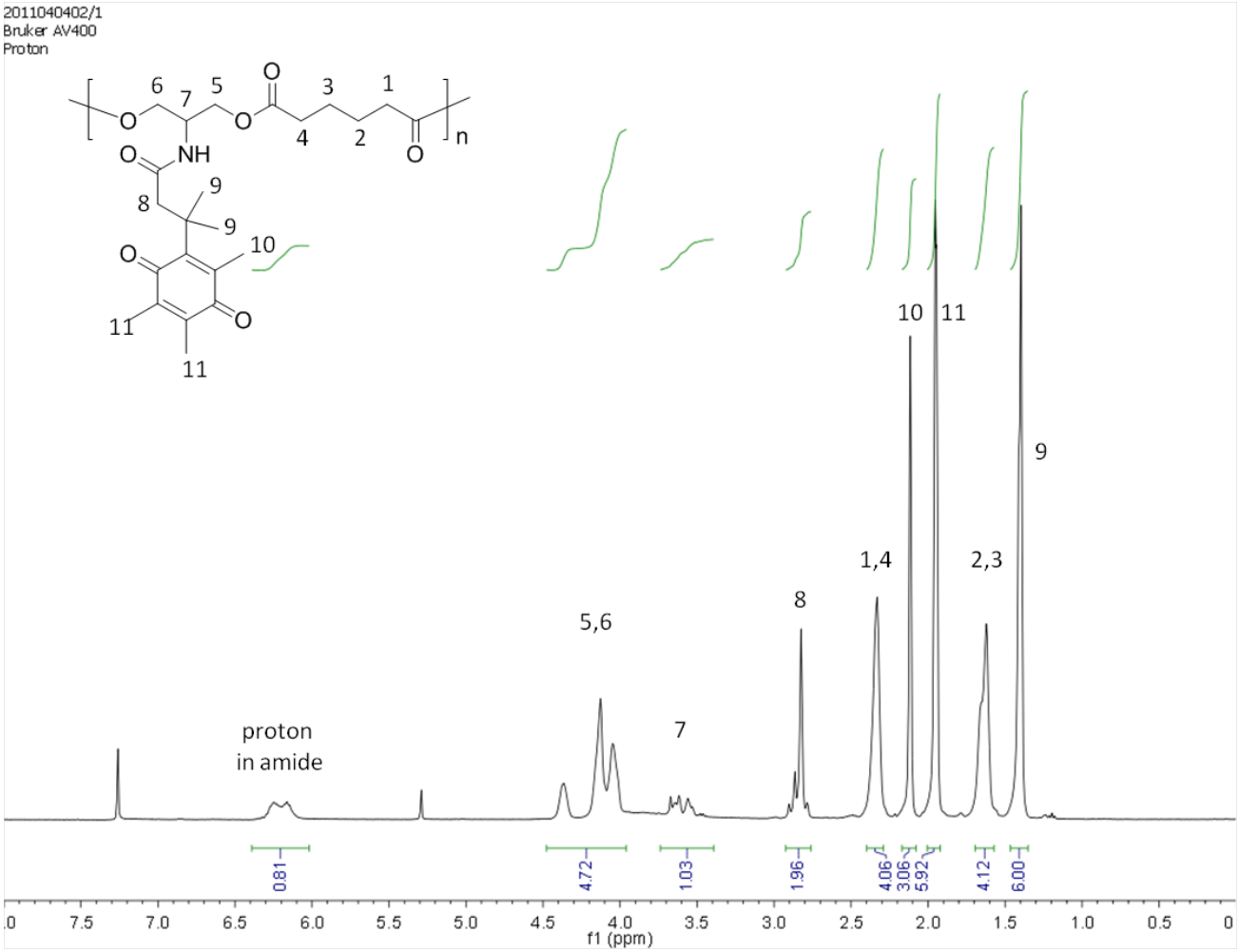


Figure 2. ^{1}H NMR spectrum of the synthesized polymer.

4. Preparation of nanoparticles by single emulsion method

For the preparation of blank redox-sensitive nanoparticles, a synthesized polymer solution (25mg) in 0.5 mL dichloromethane was added to 9.4 mL of PBS containing 0.1 mL of tween 80 as a surfactant, while stirring at room temperature. The mixture was stirred at 1000 rpm for another 10 mins and, then, emulsified by sonication for 1 min in an ice-bath with a probe sonicator. After magnetically stirring the mixture overnight at ambient temperature, the hardened nanoparticles were filtered through a 0.45 μm filter to remove large particles and rinsed with PBS three times to remove surfactant via centrifugation. The nanoparticles were lyophilized and collected as a yellow fluffy powder (17 mg, 68%). For the preparation of paclitaxel-loaded nanoparticles, 2.5 mg of paclitaxel was dissolved in the organic phase. Drug loading in the nanoparticles was determined by direct measurements of loaded paclitaxel using a HPLC system after dissolving the dried paclitaxel-loaded nanoparticles in acetonitrile. The drug loading efficiency in the polymeric nanoparticle was determined to be 77.9%. The HPLC system consisted of a binary pump (Waters 1525), a UV detector (Water 2487), and an autosampler (Water 717). Analytical column was Waters C_{18} Symmetry column (150 mm×3.9 mm I.D., 5 μm particle size) and a mixture of acetonitrile and water (55/45, v/v) was used as the eluent solvent. The flow rate was set at 1.0 mL/min with 20 μL of injection volume, and the paclitaxel was detected at an absorption wavelength of 227 nm.

5. Nanoparticle characterization

The size distribution of blank and paclitaxel-loaded nanoparticles was measured by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The polymeric nanoparticles were also characterized by transmission electron microscopy (TEM) using a Tecnai T12 microscope (FEI, Hillsboro, OR) operated at 80 kV. Each sample was sonicated for 5 mins before being mounted on a carbon coated Formvar cooper grid (400 mesh) and dried for 3 minutes. After wicking away excess solution and air drying for 1 minute, samples were then negatively stained with uranyl acetate (2% w/v) for 30 seconds prior to TEM imaging.

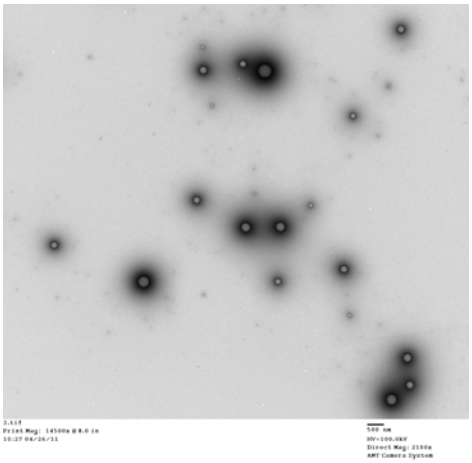


Figure 3. The TEM image of paclitaxel incorporated polymeric nanoparticles.

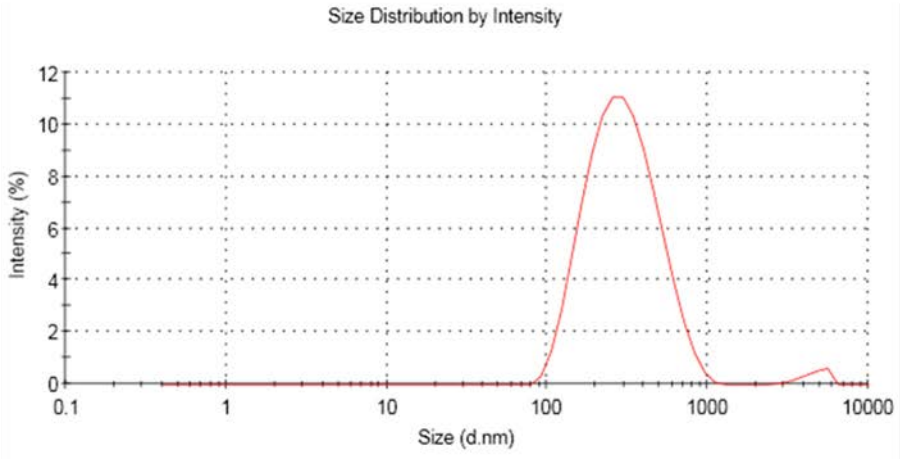


Figure 4. Size distribution and z-average diameter of the paclitaxel loaded nanoparticles

6. Paclitaxel and lactone release by chemical reduction with sodium dithionite ($Na_2S_2O_4$)

Paclitaxel release from the polymeric nanoparticles was investigated through chemical reduction by sodium dithionite *in vitro*. Drug encapsulated nanoparticles (230 μg) were suspended in PBS buffer with pH 7.4 containing sodium salicylic acid (0.8 M). After an addition of 200-fold molar excess of sodium dithionite to the benzoquinone moieties, aliquots (150 μL) were collected for the analysis with assistance of HPLC over 24 hours at appropriate time intervals, and the same volume of blank buffer media was added to maintain the total volume (3 mL). The triggered release experiment was achieved in the same manner, except the release media was incubated without sodium dithionite up to 48 hr. The 200-fold molar excess of sodium dithionite was added at 48 hrs time point, and the sample for HPLC analysis was collected essentially same manner as described in the section 4. A control solution of the nanoparticles was prepared with the same buffer solution without sodium dithionite. Since the retention times of released lactone and paclitaxel are different each other, the released lactone upon the chemical reduction was also monitored by HPLC at the same time (Figure 5). Synthesized lactone which was used for the calibration curve to determine the amounts of released lactone, compound 2 in the scheme 1, showed the same retention time as the lactone release from the polymer after reduction.

The effects of the chemical reduction on polymer structure were further examined by the analysis of resulting compounds after polymer reduction. Ten milligrams of the redox-sensitive polymer was dissolved in 1 mL of 50% v/v acetonitrile in deionized distilled water containing sodium dithionite at a 200-fold molar excess to the benzoquinone. After vigorously vortexing the mixture, the mixture was concentrated by the removal of acetonitrile under a continuous flow of nitrogen gas. After freeze drying, the concentrated mixture was extracted with acetonitrile to obtain the organic solvent-soluble products. Remaining mixture was further extracted with water to obtain water-soluble reduction products. The extracts were characterized by NMR after being dissolved either in $CDCl_3$ or D_2O. As shown in Figure 6 (a), the organic solvent-soluble portion,

represented by the top spectrum, was turned out to be the benzoquinone lactone of which spectrum is shown at the bottom. On the other hand, NMR spectrum of the water-soluble component indicated that the main water-soluble reduction product was poly(serinol adipate) as shown in Figure 1-(b). Characteristic methylene proton peaks in adipate appeared at 1.7 and 2.6 ppm while the proton peaks from serinol appeared at 4.1 and 4.5 ppm. This result also indicated that poly(serinol adipate) with free amines, resulting product after reduction of the TMBQ-based redox-sensitive polymer, is water soluble.

We have also tested the effect of sodium dithionite on paclitaxel at the same molar ratio used for the release study and confirmed that sodium dithionite did not affect paclitaxel analysis even after 24 hrs incubation as shown in Figure 7.

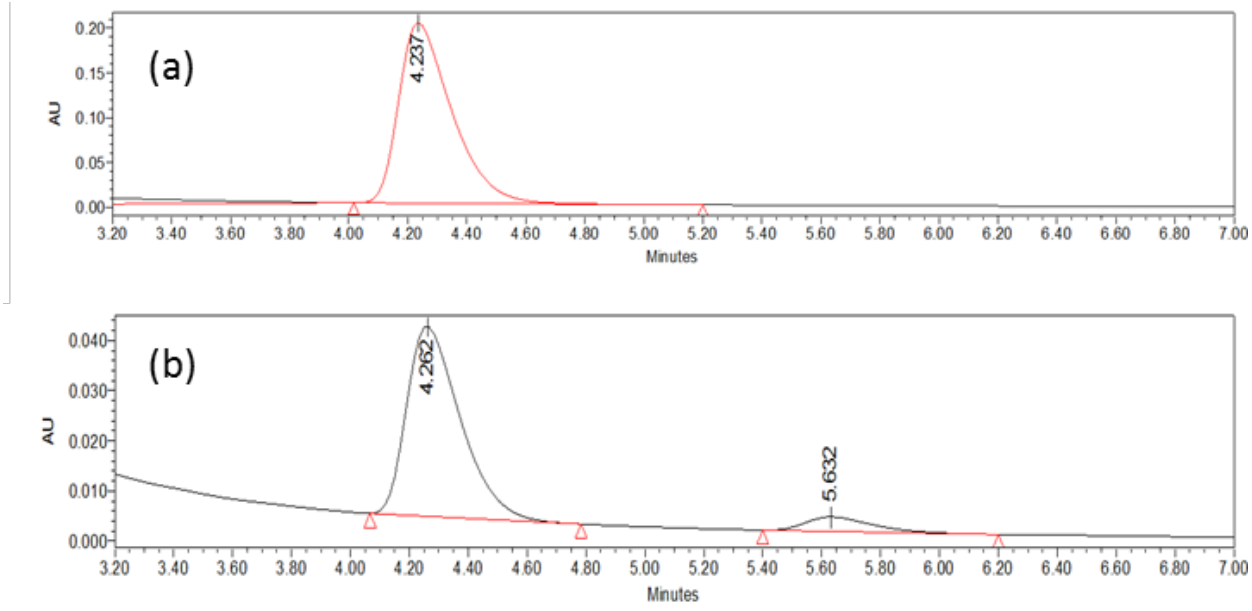


Figure 5. HPLC chromatograms of (a) the synthesized lactone and (b) a sample from the in vitro release study.

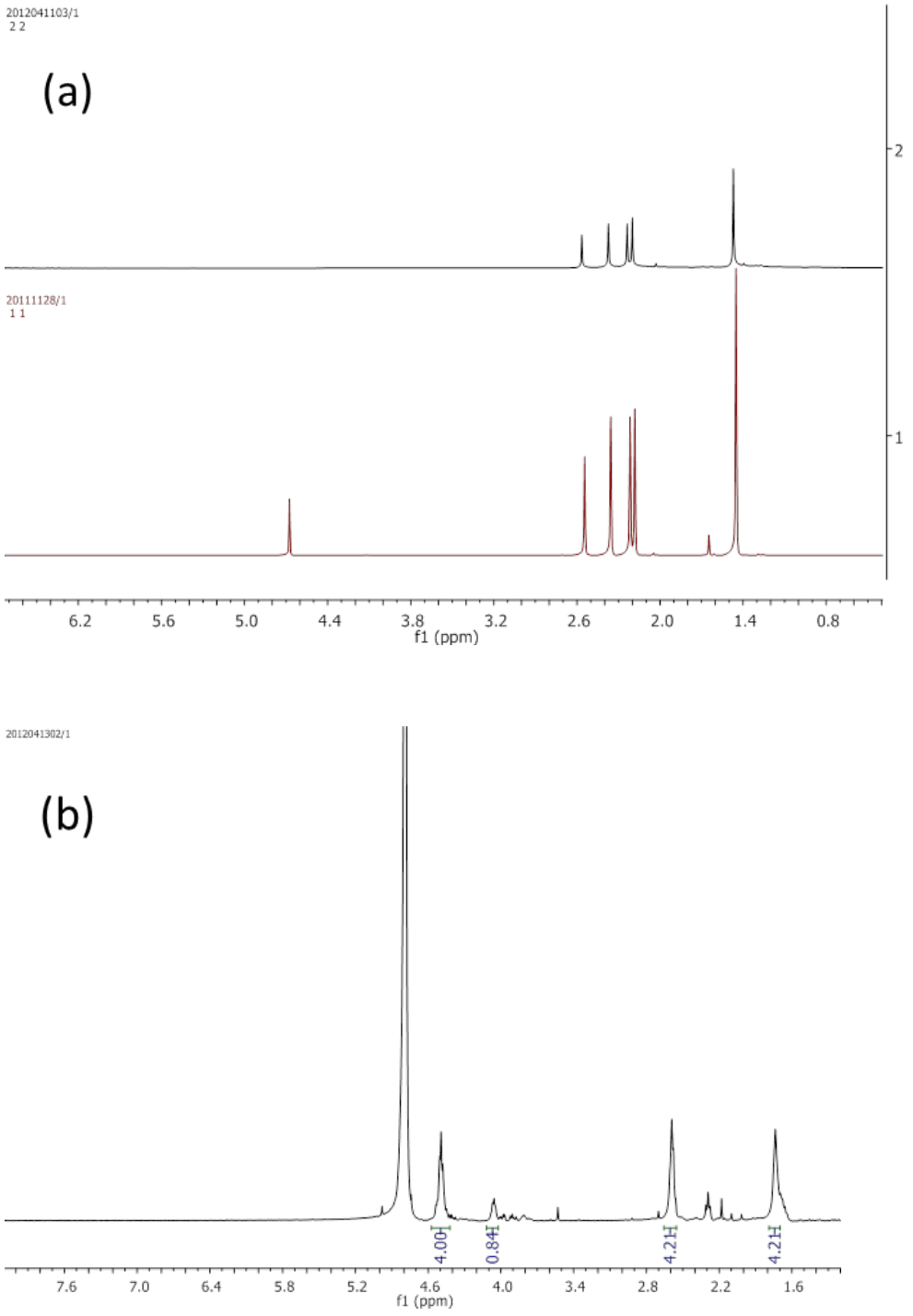


Figure 6. NMR spectra of the reduction products after polymer reduction by sodium dithionite. (a) NMR spectra of the organic solvent-soluble components after polymer reduction (top), and synthesized benzoquinone lactone (bottom) in $CDCl_3$. (b) NMR spectrum of the water-soluble components after polymer reduction in D_2O.

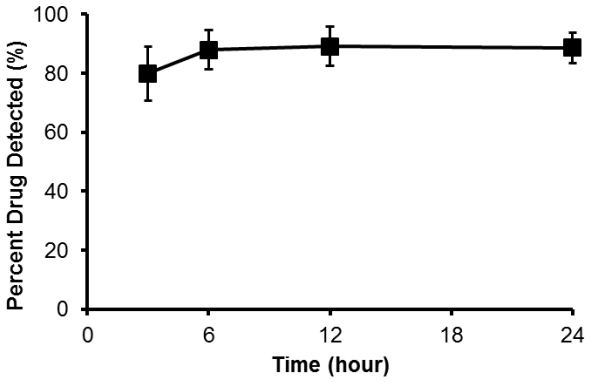


Figure 7. The effect of sodium dithionite on the detection of paclitaxel by HPLC.

7. Statistical analysis

Student's t-test used for statistical analyses of data. The differences were considered significant for p value of <0.05.